

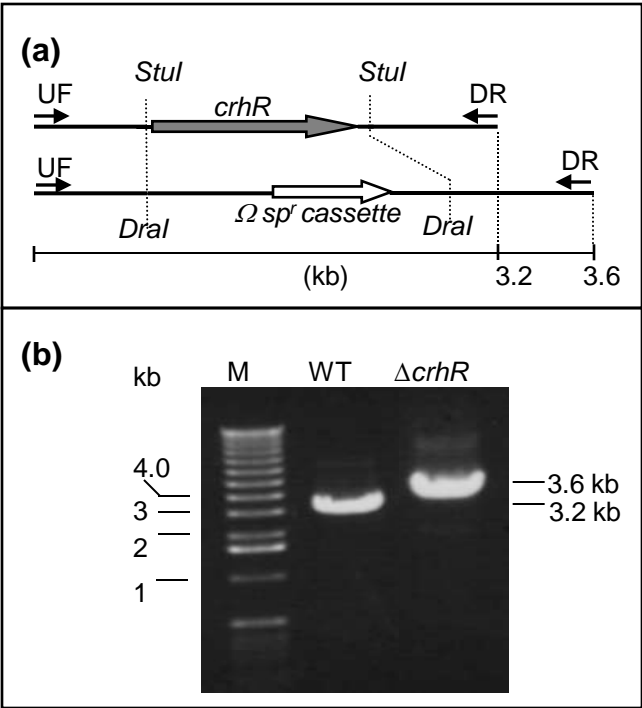


An RNA helicase, CrhR, regulates the low-temperature-inducible expression of heat-shock genes groES, groEL1 and groEL2 in *Synechocystis* sp. PCC 6803

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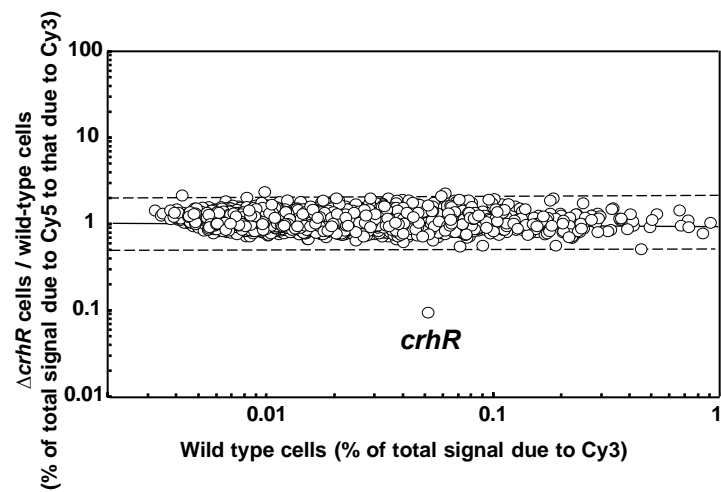
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Fig. 1S



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Fig. 2S



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Figure 3S

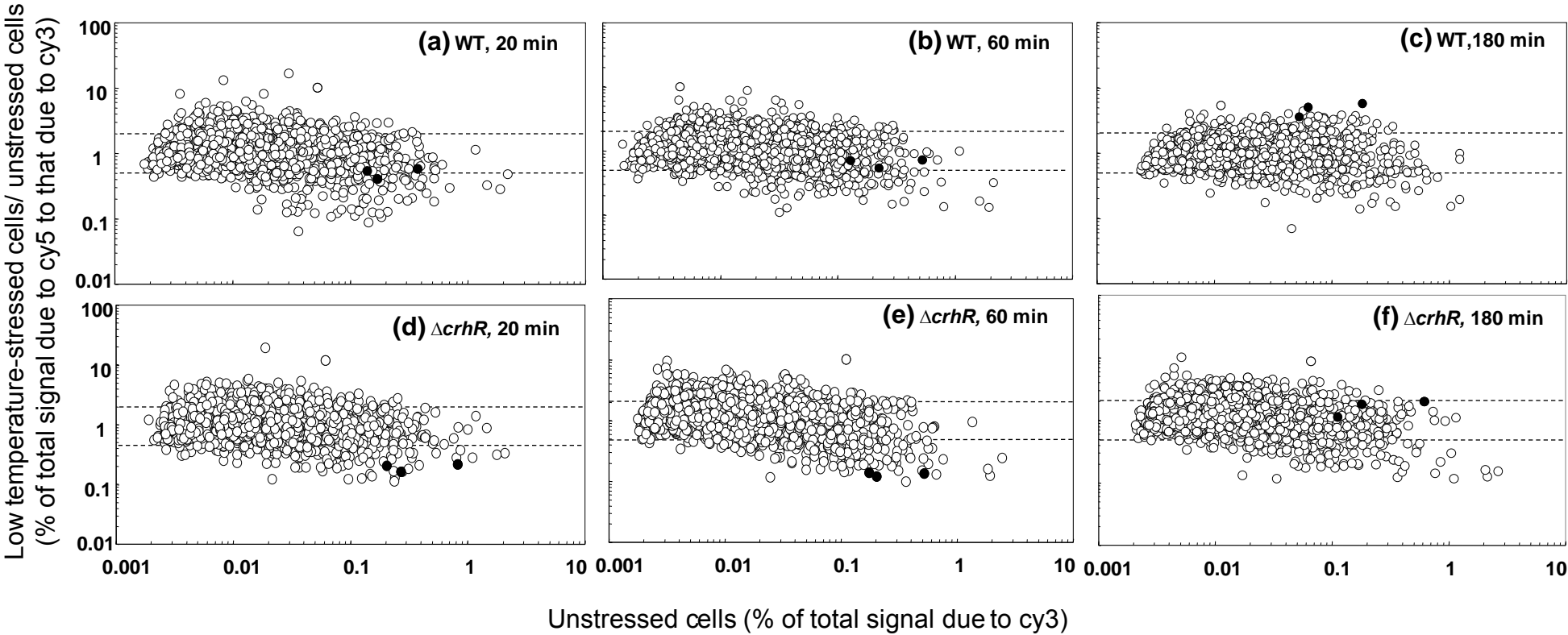


Fig. 4S

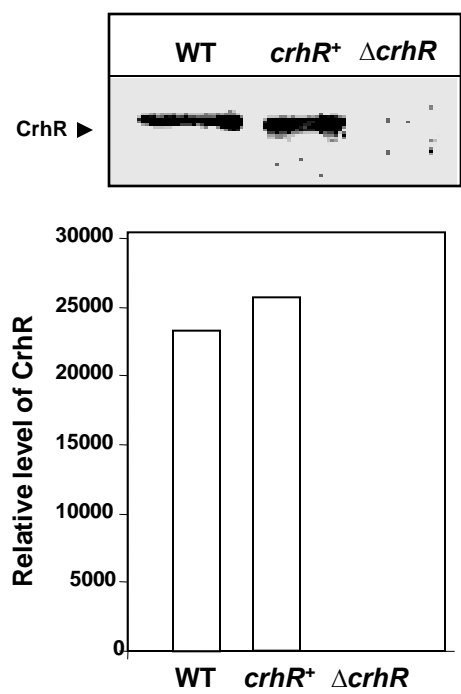
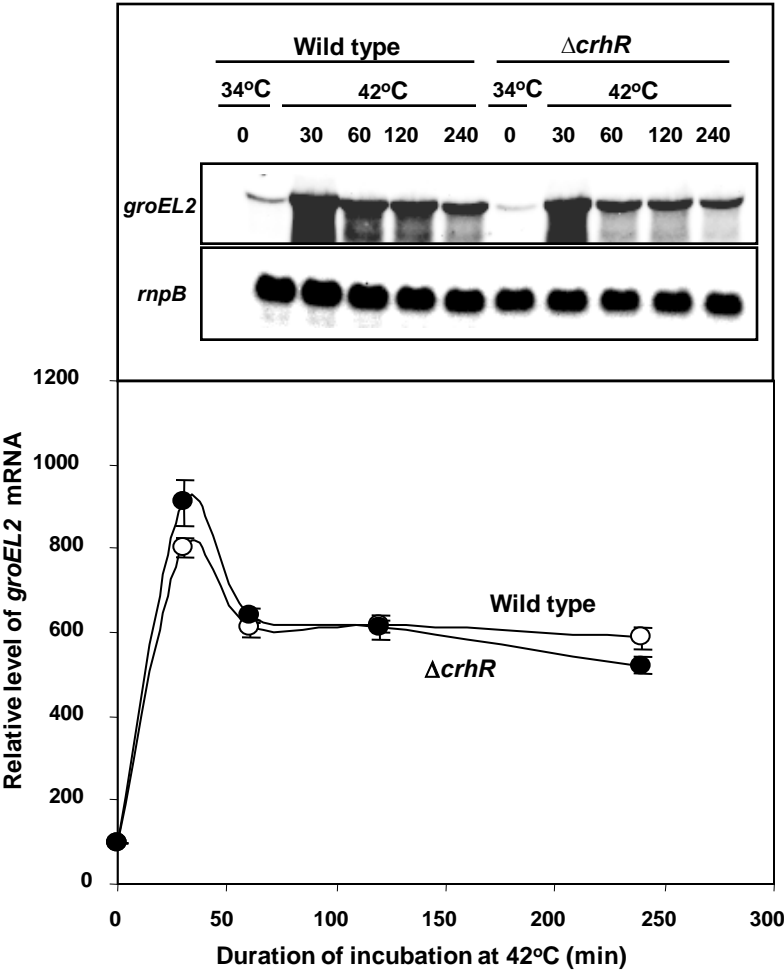


Fig. 5S



Supplementary data

Fig. 1S. Strategy for disruption of the *crhR* gene in the genome of *Synechocystis* sp. PCC 6803: The wild-type copy of the *crhR* gene was completely replaced by the mutated copy of the gene in $\Delta crhR$ mutant cells. (a) Schematic representation of the genotype of the $\Delta crhR$ mutant. The *crhR* (*slr0083*) gene and the spectinomycin-resistance gene (*sp^r*) cassette are shown in the filled and open arrows, respectively. Thick arrows indicate UF and DR primers used for PCR amplification of the wild-type copy of the *crhR* gene and that of the *sp^r* cassette. (b) Genomic PCR analysis with the primers indicated in (a). M represents 1-kb DNA ladder. Other details are described in METHODS.

Fig. 2S. DNA microarray analysis to compare the gene expression in $\Delta crhR$ mutant cells with that in wild-type cells both grown at 34°C for 16 h. RNAs extracted from wild-type and the mutant cells were used for synthesis of Cy3- and Cy-5 labeled cDNAs, respectively. Cy5 / Cy3 ratio of almost all the genes was between 2 and 0.5 (indicated by dashed lines), suggesting that the mutation in *crhR* gene had no effect on gene expression when both types of cell were grown isothermally at 34°C. The Cy5 / Cy3 ratio of the *crhR* gene appeared at 0.1, confirming the mutation in *crhR* gene. Similar results were obtained in two independent experiments, and the figure shows the result of one of these experiments.

Fig. 3S. DNA microarray analysis of the low temperature-induced expression of genes in wild-type and $\Delta crhR$ mutant cells. (a), (b), and (c) represent that the expression of wild-

type (WT) cells that had been grown at 34 °C for 16 h and then incubated at 24 °C for 20, 60, and 180 min (low temperature stressed) was compared with that of wild-type cells that had been grown at 34 °C (control cells). (d), (e), and (f) represent that the gene expression in $\Delta crhR$ mutant cells, which had been grown at 34 °C for 16 h and then incubated at 24 °C for 20, 60, and 180 min (low temperature stressed), were compared with that in $\Delta crhR$ mutant cells, which had been grown at 34 °C (control cells). RNAs extracted from control cells and low temperature-stressed cells were used for synthesis of Cy3- and Cy-5 labeled cDNAs, respectively. Closed circles correspond to *groES*, *groEL1* and *groEL2* genes. The dashed lines represent the genes whose expression was induced and repressed. Similar results were obtained in two independent experiments, and the figure shows the result of one of these experiments.

Fig. 4S. Levels of CrhR in wild-type, *crhR*⁺ and $\Delta crhR$ cells. Soluble proteins were extracted from the three kinds of cell that had been grown at 34 °C and then incubated at 24°C for 180 min. Samples equivalent to 25 µg of proteins were loaded in each well. Similar results were obtained in two independent experiments, and the figure shows the result of one of these experiments.

Fig. 5S. Northern blotting analysis of the heat-induced expression of the *groEL2* gene in wild-type and $\Delta crhR$ cells. RNAs were extracted from wild-type and $\Delta crhR$ cells that had been grown at 34 °C for 16 h and then incubated at 42 °C for 30, 60, 120 and 240 min. Aliquots (15 µg) of the extracted RNA were fractionated on 1.2% agarose gels that contained 1.4 M formaldehyde. A 1646 bp DNA fragment that included the *groEL2* gene

was amplified using primer set 5'TTT CCT TTA AGG ATG AAT CCA G3' and a reverse primer 5'TTA CATCAT GCC CAT GCC3' by PCR and used as a probe. ○, Wild-type cells; ●, $\Delta crhR$ cells. For normalization, extents of signals obtained with *groEL2* mRNA were compared with signals of *rnpB* mRNA. Experiment was performed three times and the results are presented as a mean of the three independent experiments.